# CHARM SCIENCES, INC.
# ROSA WET FUMONISIN QUANTITATIVE TEST

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## GENERAL INFORMATION

ROSA WET Fumonisin Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology and Water Extraction Technology (WET) that eliminates the use of organic solvents (methanol, ethanol, etc.). WET uses a non-hazardous extraction powder added to the sample followed by water (distilled or deionized) to extract fumonisins into the aqueous solvent. Fumonisin interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the ROSA-M Reader and interpreted as parts per billion (ppb) or parts per million (ppm) Fumonisin. To convert results in ppb to ppm divide by 1000 (e.g., 5000 ppb = 5 ppm).

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@udsa.gov.

**Approved Test Kit Information**

| | |
|---|---|
| **Test Kit Vendor:** | *Charm Sciences, Inc.* 978-687-9200 |
| **Test Kit Name:** | ROSA WET Fumonisin Quantitative Test |
| **Product Number:** | LF-FUMQ-WET |
| **Effective Date of Instructions:** | 2/2/2015 |
| **Instructions Revision Number** | 0 |
| **Conformance Range:** | 0.5 – 5.0 ppm |
| **Number of Analyses to Cover Conformance Range:** | 2 |
| **Type of Service:** | Quantitative |
| **Supplemental Analysis:** | Yes |
| **Approved Commodities:** | Corn, barley, millet, oats, popcorn, rough rice, sorghum, and wheat |
| **Extraction method:** | Shake vigorously 50 grams ground sample with the contents of one (1) packet WET Extraction Powder and 150 milliliters (mL) of deionized or distilled water for 1.5 minutes. For barley, shake vigorously 50 grams ground sample with the contents of two (2) packets WET Extraction Powder and 250 mL of deionized or distilled water for 1.5 minutes. |
| **Test Format:** | Lateral flow strip |
| **Detection Method:** | ROSA-M Reader, Model LF-ROSAREADER-M-NB |

## PREPARATION OF TESTING MATERIALS AND EQUIPMENT

**a. Test Strips:**

Remove from the container only the number of test strips to be used in 1 day, document time of removal. Keep these test strips at room temperature during daily use for up to 12 hours and unused test strips should be discarded.

**b. FUMQ-W Dilution Buffer:**

(1) Dispense buffer into a clean micro-centrifuge tube and label for each sample to be tested.

(2) Use pre-dispensed buffer tubes and buffer solution at room temperature (18 to 30 °C).

**c. Negative Control:**

FUMQ-W Dilution Buffer is used as a negative control in Test Procedures section.

**d. Positive Control:**

Reconstitute the dry positive control (provided with test kit) by adding 3.0 mL FUMQ-W Dilution Buffer. Shake well; allow to stand for 10 minutes at room temperature before use, and mix again just before use in Test Procedures section.

**e. Reader and Test Strip Performance Testing:**

(1) Equipment Setup

**ROSA-M Reader:** Enter performance mode in ROSA-M Reader by selecting FUM channel in 3-line mode (FUM flashing) and sequentially pressing ESC, 5, ENTER. Follow ROSA-M Reader prompts to test calibration strips (LOWCAL and HIGHCAL) and controls (NEGCONTROL and POSCONTROL).

(2) Test calibration strips daily to verify ROSA-M Reader performance. Calibration strips must test/perform in the specified ranges.

(3) Test negative control and positive control weekly to verify test strip performance. Valid control ranges are:

(a) Negative Control: less than 100 ppb (0.1 ppm)

(b) Positive Control: 500 to 1100 ppb (0.5 to 1.1 ppm)

**If calibration strips or controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance. Notify your monitoring field office or TSD with any documented information for quality control purposes.**

**f. ROSA Incubator:**

ROSA Incubator must be clean and level. The ROSA Incubator temperature must be at 45 ± 1 °C (the temperature indicator should match the incubator temperature).

## EXTRACTION PROCEDURES

**a. Procedure for corn, millet, oats, rough rice, and wheat:**

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.

(2) Add contents of one (1) packet WET Extraction Powder for a 50 gram ground sample.

(3) Add 150 mL deionized or distilled water.

(4) Shake vigorously for 1.5 minutes.

(5) Allow sample to settle for 1 minute to obtain settled extract (use within 30 minutes).

(6) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).

(7) Repeat steps 1 to 6 for additional samples.

**b. Procedure for popcorn and sorghum:**

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.

(2) Add contents of one (1) packet WET Extraction Powder for a 50 gram ground sample.

(3) Add 150 mL deionized or distilled water.

(4) Shake vigorously for 1.5 minutes.

(5) Allow sample to settle for 1 minute to obtain settled extract (use within 30 minutes).

(6) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).

(7) Filter centrifuged extract by drawing into syringe and passing through GF/CA filter (purchased separately). Collect filtered extract in a clean micro-centrifuge tube and label.

(8) Repeat steps 1 to 7 for additional samples.

**c. Procedure for barley:**

(1) Weigh 50 ± 0.2 grams ground samples into a clean extraction container.

(2) Add contents of two (2) packets WET Extraction Powder for a 50 gram ground sample.

(3) Add 250 mL deionized or distilled water.

(4) Shake vigorously for 1.5 minutes.

(5) Allow sample to settle for 1 minute to obtain settled extract (use within 30 minutes).

(6) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (use within 2 hours).

(7) Repeat steps 1 to 6 for additional samples.

## SAMPLE PREPARATION FOR QUANTIFICATION

**a. <u>Sample Preparation for corn, millet, popcorn, oats, rough rice, sorghum, and wheat:</u>**

(1) Preparation of Diluted Extract for 0.5 to 1.5 ppm quantitation.

(a) Pipet 900 microliters (µL) FUMQ-W Dilution Buffer into a clean micro-centrifuge tube.

(b) Pipet 100 µL centrifuged or filtered sample extract to micro-centrifuge tube containing 900 µL FUMQ-W Dilution Buffer, cap, mix (5 times inverting up and down), and label. This sample is the **Diluted Extract**, and ready for the test (use within 6 hours after preparation).

(c) Repeat for additional samples.

(2) Preparation of Second Diluted Extract for 1.0 to 5.0 ppm quantitation.

(a) Pipet 900 µL FUMQ-W Dilution Buffer into a clean micro-centrifuge tube.

(b) Pipet 100 µL Diluted Extract to micro-centrifuge tube containing 900 µL FUMQ-W Dilution Buffer, cap, mix (5 times inverting up and down), and label. This sample is the **Second Diluted Extract**, and ready for the test (use within 6 hours after preparation).

(c) Repeat for additional samples.

**b. <u>Sample Preparation for barley:</u>**

(1) Preparation of Diluted Extract for 0.5 to 1.5 ppm quantitation.

(a) Pipet 1000 µL (1.0 mL) FUMQ-W Dilution Buffer into a clean micro-centrifuge tube.

(b) Pipet 200 µL centrifuged sample extract to micro-centrifuge tube containing 1000 µL FUMQ-W Dilution Buffer, cap, mix (5 times inverting up and down), and label. This sample is the **Diluted Extract**, and ready for the test (use within 6 hours after preparation).

(c) Repeat for additional samples.

(2) Preparation of Second Diluted Extract for 1.0 to 5.0 ppm quantitation.

(a) Pipet 900 µL FUMQ-W Dilution Buffer into a clean micro-centrifuge tube.

(b) Pipet 100 µL Diluted Extract to micro-centrifuge tube containing 900 µL FUMQ-W Dilution Buffer, cap, mix (5 times inverting up and down), and label. This sample is the **Second Diluted Extract**, and ready for the test (use within 6 hours after preparation).

(c) Repeat for additional samples.

**NOTE: Laboratories may initially test the Second Diluted Extract if levels typically reported in their market area are within the 1.0 to 5.0 ppm testing range.**

## TEST PROCEDURES

**a. <u>Sample Analysis:</u>**

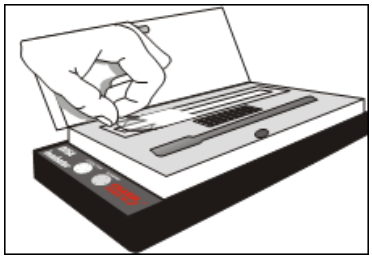
(1) Check that the ROSA Incubator temperature is 45 ± 1 °C.

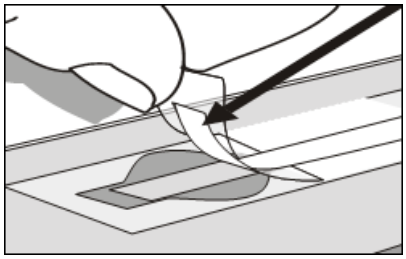
(2) Label test strip(s) to identify sample.

(3) Place test strip in the ROSA Incubator with the flat side facing upward.

(4) Hold the test strip flat in the ROSA Incubator and use tab to expose sample compartment by peeling tape back to "Peel to Here" line.

Avoid lifting the test strip and sponge under tape and bending back the white wick and sponge under the tape.





(5) Hold the pipet vertically and slowly pipet 300 µL test sample (diluted extract or positive and negative control) into the sample compartment at the ROSA Incubator line.

(6) Reseal the tape over the sample pad compartment.

**NOTE:** When performing multiple tests using a ROSA Incubator:

(a) Peel, pipet, and reseal before starting next strip.

(b) Complete all test strips within 1 minute.

(7) Close lid on the ROSA Incubator.

(8) Incubate for 5 minutes.

(9) Remove strip from the ROSA Incubator.

Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until interpreted.

(a) Wipe foreign matter (dust, etc.) from the test strip(s).

(b) Inspect and read test strip(s) within 2 minutes of incubation completion. When running multiple test strips in the ROSA Incubator, remove one strip for visual inspection and interpretation at a time.

(c) Lower ROSA Incubator lid; do not re-latch.

**b. <u>Visual Inspection:</u>**

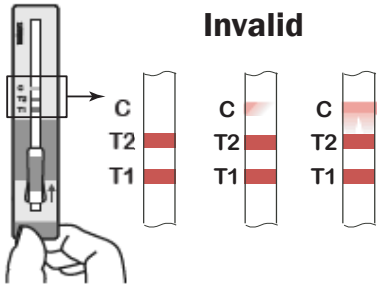
(1) The test strip is **INVALID** if any of the following are observed:

(a) C (Control) line is missing.

(b) T1, T2 (Test) or C line is smeared or uneven.

(c) T1, T2, or C line is obscured by diluted extract or control.

(d) Beads do not flow past T1, T2 or C lines.




(2) Do not put INVALID test strips in the Charm EZ-M reader.

(3) If test strip is INVALID, re-test the diluted extract or control.

**c. <u>Interpretation:</u>**

(1) Insert a clean and valid test strip into the ROSA-M Reader. Slide the strip into the slot with the sample compartment in the down position until it stops.


(2) Read results on FUM channel in 3-line mode (FUM flashing) using the appropriate MATRIX. If desired, enter Sample and/or Operator. Press ENTER to read.

- **MATRIX 00:** Diluted Extract for 0.5 to 1.5 ppm quantitation.
- **MATRIX 01:** Second Diluted Extract for 1.0 to 5.0 ppm quantitation.
- **MATRIX 02:** Supplemental Diluted Extract for 1 to 5 ppm (Uncorrected Fumonisin Concentration) quantitation.

**For controls, see Reader and Test Strip Performance Testing in Preparation of Testing Materials and Equipment section.**

(3) **READING:** The number displayed is the concentration of fumonisins (ppb or ppm) in the sample. A reading in ppb must be converted to ppm by dividing the ppb concentration by 1000 (e.g., 500 ppb = 0.5 ppm).

A "+" sign on a READING value indicates that the concentration of the sample is greater than the Sensitivity range. For example, a Diluted Extract READING of "+1500 ppb" or "+1.5 ppm" indicates a value greater than 1.5 ppm. For quantitation of 1.0 to 5.0 ppm fumonisins, prepare the Second Diluted Extract and use with another test strip.

A Second Diluted Extract READING less than 1.0 ppm indicates a value below the detection range. Re-test Diluted Extract on another test strip for quantitation from 0 to 1.5 ppm fumonisins.

A Second Diluted Extract READING greater than 5.0 ppm indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. An applicant can request a supplemental analysis option to report test results above the Second Diluted Extract sensitivity range of 5.0 ppm. See Supplement Analysis procedures for more information.

**Note: Applicants may request qualitative certification in lieu of retesting of results outside of the Diluted or Second Diluted Extract test sample sensitivity ranges/concentrations.**

## SUPPLEMENTAL ANALYSIS

Supplemental analysis is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation.

The range for performance evaluation of quantitative fumonisins test kits is 0.5 to 5.0 ppm. Therefore, supplemental analysis would be performed for a result above 5.0 ppm. In supplemental analysis, the Second Diluted Extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range, and a correction for dilution is applied to derive the final result. For this test kit, the READING is an Uncorrected Fumonisin Concentration in the sample and the Corrected Fumonisin Concentration is obtained by multiplying the Uncorrected Fumonisin Concentration by the dilution factor used to prepare the Supplemental Diluted Extract.

Supplemental analysis is performed only at the request of the applicant.

**Preparation and Assay of Supplemental Diluted Extract.**

(1) Prepare Second Diluted Extract according to Sample Preparation for Quantification.

(2) Determine and record the Dilution Factor (DF) required to prepare Supplemental Diluted Extract for the Suspected Sample Concentration. The Dilution Factor (see equation below) is equal to the sum of the volume of the FUMQ-W Dilution Buffer plus the volume of the Second Diluted Extract divided by the volume of the Second Diluted Extract. See table below for examples.

$$\mathbf{DF} = \frac{\text{Dilution Buffer Volume (in µL)} + \text{Second Diluted Extract Volume (in µL)}}{\text{Second Diluted Extract Volume (in µL)}}$$

| DF | FUMQ-W Dilution Buffer Volume | Second Diluted Extract Volume | Suspected Sample Concentration |
|---|---|---|---|
| 4 | 300 µL | 100 µL | 4 to 20 ppm |
| 10 | 900 µL | 100 µL | 10 to 50 ppm |

(3) Prepare Supplemental Diluted Extract from the Second Diluted Extract.

(a) Pipet determined volume of FUMQ-W Dilution Buffer into a clean micro-centrifuge tube.

(b) Pipet the determined volume of Second Diluted Extract to micro-centrifuge tube containing FUMQ-W Dilution Buffer, cap, mix (5 times inverting up and down), and label. This sample is the Supplemental Diluted Extract.

(4) Repeat steps 1 to 3 for additional samples.

(5) Use Supplemental Diluted Extract as test sample in Sample Analysis found in Test Procedures section.

(6) Inspect and interpret the test strip as directed in Test Procedures section.

Valid Supplemental Diluted Extract READING must be within 1.0 to 5.0 ppm detection range of the sample dilution. Multiply the result by the Dilution Factor used to prepare the Supplemental Diluted Extract to convert the Uncorrected Fumonisin Concentration to the final Corrected Fumonisin Concentration.

**Example:** If the Uncorrected Fumonisin Concentration is 2.0 ppm and the Dilution Factor is 4 the final Corrected Fumonisin Concentration is 8 ppm (2.0 ppm x 4 = 8 ppm).

(7) A final result less than 3.05 ppm is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Second Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 5.0 ppm.

A reading greater than 5.0 ppm (uncorrected fumonisins concentration) indicates that the concentration of the sample is greater than the test range. Prepare another Supplemental Diluted Extract with a higher Dilution Factor and run another test strip to quantitate.

## REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

## STORAGE CONDITIONS AND PRECAUTIONS

**a. Storage Conditions:**

(1) Store test strips refrigerated in tightly closed supplied container.

(2) Store dilution buffer bottle and pre-dispensed micro-centrifuge tubes refrigerated.

(3) Store reconstituted positive control refrigerated (0 to 7 °C) for up to 1 week or aliquot (at least 0.5 mL) to clean micro-centrifuge tubes, label, and freeze within 6 hours of reconstitution (-15 °C or below) for up to 2 months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed positive control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.

**b. Precautions:**

(1) Test Strips

(a) To open test strip canister, remove and save plastic lid with foil lined foam insert to reseal container. Lift foil tab and peel foil seal off container. Discard foil seal.

(b) In high humidity, limit condensation by opening container after it has warmed to room temperature, estimated between 20 to 30 minutes from the time the container was removed from the refrigerator.

(c) Inspect/verify desiccant indicator. Beads inside desiccant packets should be blue. Do not use test strips if the blue beads have turned purple or pink

(2) Use FUMQ-W Dilution Buffer supplied with each test kit only.

(3) Do not use the test kits beyond the noted expiration date.

(4) Debris on test strips may alter the reader optics. Keep equipment clean. Wipe dust and liquid off test strips before inserting into reader.

(5) ROSA Incubator must be clean and level. ROSA Incubator temperature must be 45 ± 1 °C. The temperature indicator should match the ROSA Incubator temperature. A daily thermometer check is recommended. Keep ROSA Incubator lid lowered, but not latched unless performing test procedure. ROSA Incubator may take 10 minutes to reach proper temperature depending on ambient temperature.

## EQUIPMENT AND SUPPLIES

**a. Test Strips**

(1) LF-FUMQ-WET-20K

(a) 1 container of 20 FUMQ-WET test strips

(b) 1 Fumonisin B1 Positive Control

(c) 1 FUMQ-W Dilution Buffer

(2) LF-FUMQ-WET-100K

(a) 1 container of 100 FUMQ-WET test strips

(b) 1 Fumonisin B1 Positive Control

(c) 1 FUMQ-WET Dilution Buffer

(3) LF-FUMQ-WET-500K

(a) 5 containers of 100 FUMQ-WET test strips

(b) 5 Fumonisin B1 Positive Controls

(c) 5 FUMQ-W Dilution Buffers

**b. Materials required but not provided**

(1) 100 µL pipet and pipet tips

(2) 300 µL pipet and pipet tips

(3) 100 to 1000 µL variable volume pipet and pipet tips

(4) 250 mL graduated cylinder

(5) Balance

(6) Deionized or distilled water

(7) Micro-centrifuge tubes

(8) Mini-centrifuge

(9) ROSA-M Reader

(10) Printer for ROSA-M Reader (optional)

(11) ROSA Incubator

(12) Sample extraction containers

(13) Sample grinder

(14) Storage bottle

(15) Transfer pipets (optional)

**c. <u>Extraction Powder required for testing, purchased separately</u>**

(1) LF-WET-EXT-50G-20: WET Extraction powder for 50 gram sample (20/pack)

(2) LF-WET-EXT-50G-100: WET Extraction powder for 50 gram sample (100/pack)

**d. <u>Materials required but not provided for popcorn and sorghum</u>**

(1) GF/CA syringe filters (Phenomenex Part No. AF0-8A09-12)

(2) Syringes

## REVISION HISTORY

Revision 0 (2/2/2015)